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(54) METHODE DE DETECTION DES SYMPTOMES ET DE DIAGNOSTIC D'ENCEPHALOPATHIES SPONGIFORMES TRANSMISSIBLES

(54) DETECTION AND DIAGNOSIS OF TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

(57)

In the present invention, we described the use of anti-DNA antibody for the detection of prions and diagnosis of Transmissible Spongiform Encephalopathies (TSE) diseases in animals and humans.

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Abstract

In the present invention, we described the use of anti-DNA antibody for the detection of prions and diagnosis of Transmissible Spongiform Encephalopathies (TSE) diseases in animals and humans.

Detection and Diagnosis of Transmissible Spongiform Encephalopathies

By
Jian Zheng and Steve Alexander

Field of the Invention

In the present invention, we described the use of anti-DNA antibody for the detection of prions and diagnosis of Transmissible Spongiform Encephalopathies (TSE) diseases in animals and humans.

Background of the Invention

Transmissible spongiform encephalopathies (TSEs) comprise a group of rapidly progressing, neurodegenerative fatal diseases that affect both humans and animals. TSEs have clinical and neuropathological characteristics which include devastating dementia, pyramidal and extrapyramidal signs with myoclonus, multifocal spongiform changes, astrogliosis, amyloid plaques, neuronal loss, absence of inflammatory reaction and are usually characterized by a long incubation period.

In animals, a commonly known example of TSE disease recognized for over 200 years, is scrapie, which is found in sheep and goats (McGowan 1922). Other animal TSE diseases have also been described, such as transmissible mink encephalopathy (TME, Marsh 1976), chronic wasting disease of mule deer and elk (CWD, Williams 1980), bovine spongiform encephalopathy (BSE, commonly known as "mad-cow" disease (Wells 1987), and the more recently described feline spongiform encephalopathy of domestic cats, pumas, and cheetahs (Wyatt 1991).

In humans, TSEs have been traditionally classified into Creutzfeldt-Jakob disease (CJD), kuru, Gerstmann-Sträussler-Scheinker syndrome (GSS) and fatal familial insomnia (FFI). Among them, Kuru has been described only in the Fore linguistic group of New Guinea. For many years after its first recognition in 1957, kuru was the most common cause of death among women in the affected population, but its occurrence has declined because of the cessation of cannibalism that had facilitated disease transmission. As of today, only a few cases still occur due to the long incubation periods typical of this condition.

Although these rare neurodegenerative disorders occur in about 0.5 to one person per million worldwide (Brown 1987), TSEs attracted considerable public attention because of the unique biology and concerns about a onset of the epidemic of a newly recognized bovine spongiform encephalopathy (BSE) and its potential effects on human. There is mounting evidence that through dietary exposure to BSE infected tissues, it has poses a serious threat to public health and has resulted in an increased number of incidents of a newly recognized variant form of CJD (vCJD). Until now, there have been more than 100 cases of vCJD reported, a majority which are located in UK.

It is believed that prions are the pathogenic agent causing TSE. Many efforts have been directed towards identifying the etiological agent that causes TSEs. Early on, the transmissibility of TSE disease had been experimentally demonstrated in many cases, kuru and CJD from humans to chimpanzees (Gajdusek 1966, Gibbs 1968), transmissible scrapie from sheep to sheep (Cuillé 1936) and across species to goat (Pattison 1957). The most significant breakthrough was the successful transmission of scrapie to mice, by Richard Chandler in 1961 (Chandler 1961). Chandler's discovery greatly facilitated TSE research by providing an experimental model that was cheaper and easier to manipulate. Although all of the above modes of transmission were demonstrated experimentally, the cause of recent BSE in cattle and new variant CJD in human (vCJD) was considered a consequence of dietary exposure to the mix of scrapie sheep carcasses rendered for animal feed in the case of BSE (Brown 1997), and to beef from cattle affected with BSE in the case of vCJD (Bruce 1997).

It was suggested that TSE diseases might be caused by "slow viruses" or viroids (Gajdusek 1977). However, the extreme resistance of scrapie infectivity to radiation, nucleases, and other reagents damaging to genetic materials are inconsistent with the "virus" theory. Moreover, the infectious TSE agent

could tolerate very high levels of heat and high concentrations of formaldehyde (Pattison 1965) while still able to replicate with the 'incubation period' varying from a few months to over a year (Alper 1966).

All these "unusual" characteristics of the TSE infectious agent led Dr. Stanley Prusiner to propose the concept of "prions" in 1982 (Prusiner 1982). Prion (PrP), which stands for nucleic acid-free *pro*teinaceous *in*fectious particle, is a glycoprotein present in humans and animals. In humans, it is encoded by PRNP on chromosome 20 (Robakis 1986). The cellular form of this protein (PrP^C) has two N-link glycosylation sites and a GPI anchor at the C-terminus. It has been most commonly found in neurons, and, to a much lower extent, it has also been found in other cells such as leucocytes, monocytes and platelets (Holada 2000). Furthermore, a soluble form of PrP that lacks the glycolipid anchor was detected in murine and human serum. The transmissible scrapie disease form of the prion protein (PrP^{Sc}) is a protease resistant isoform of its cellular precursor and is predominantly found in brain. At much lower level, it has also been found in tonsil, spleen, and lymph nodes in vCJD patients (Parizek 2001). The conversion from PrP^C to PrP^{Sc} is believed to be accomplished through a conformational change within the protein. Although there is still ambiguity concerning the mechanism of the conversion, much experimental evidence indicates that in the presence of PrP^{Sc}, normal PrP^C, acting as a substrate, undergoes a conformational structure change, and becomes PrP^{Sc}. This process of propagation involves replicating the conformation of PrP^{Sc} in PrP^C and results in PrP^{Sc} aggregation and amyloid rod formation, hence causing cell death (Hope 1986, Horwich 1997). As a result of Prusiner's concept of the "prion" as an infectious agent responsible for scrapie disease, and by extension, that of all TSE diseases gave rise to the notion of what are commonly referred to as Prion diseases to describe a class of pathologies believed to be linked to this protein.

Characteristics of PrP^C and PrP^{Sc}

The major property that differentiates PrP^C and PrP^{Sc} is their distinct conformation. The structural change from PrP^C to PrP^{Sc} is most supported by a crucial conformational change, involving a substantial increase in the amount of □-sheet structure of the protein, with possibly a small decrease in the amount of □-helix, indicated by circular dichroism and infrared spectroscopy (Pan 1993, Caughey 1991). The solution structure of a fragment of the mouse PrP^C has allowed a direct determination of secondary structure content of a portion of PrP^C (121-231) by NMR (Riek 1996).

Protease resistance is another characteristic that distinguishes PrP^{Sc} from PrP^C. In cultured cells and brain or in samples from many patients with GSS, PrP^{Sc} is smaller than its cellular precursor PrP^C. Even though cellular prion and scrapie prion are two isoform of same *PRNP* genomic product, PrP^C is completely degraded by Proteinase K treatment while PrP^{Sc} undergoes only limited digestion. The digestion yields a form of protein referred to as PrP 27-30 in which the N-terminus has been removed. PrP 27-30 has been postulated to be the PrP^{Sc} core required for PrP^C hosted PrP^{Sc} replication. The protease treated prion molecule, PrP 27-30 or PrP^{res}, is tightly linked to scrapie infectivity (Gabizon 1988), and provides additional evidence that PrP^{Sc} is an infectious protein.

An additional attribute, perhaps linked to the significant increase in β-sheet structure and concomitant protease-resistance, is the observed difference in solubility between PrP^{Sc} and PrP^C. While PrP^C is a soluble protein, the PrP^{Sc} isoform is highly insoluble. Furthermore, PrP^C is found attached to the surface of neurons through a GPI tail anchored into membrane (Shyng 1994) while PrP^{res} is found in the cytoplasm of affected cells (Taraboulos 1990), most likely associated with late endosomal and lysosomal compartments (Arnold 1995), and PrP^{Sc} is also localized in amorphous aggregates in enriched fractions from infected brain (Meyer 1986). Interestingly, a disease –associated mutant PrP, the $PrP^{159stop}$ mutant was found exclusively in nucleus (Lorenz 2002).

There is mounting evidence indicating a tight linkage between scrapie infectivity and PrP 27-30. Even in the purest samples, the estimated ratio of PrP molecules to infectious units is ~10^4 to 10^5 (Horwich 1997, Bolton 2001). At such low levels of infectivity, it is possible that other components, co-factors, or covalent modifications, are required for infectivity. The transgenic studies on the susceptibility of mice expressing chimeric human-mouse PrP^C suggest the presence of at least one host factor other than PrP^C, tentatively termed factor X, which might function as a molecular chaperone in the formation of PrP^{Sc} (Telling 1995).

Other Molecules Associated with Prion Pathogen

About 15 to 20 strains of scrapie have been identified based on their incubation period and lesion patterns in the inbred mice. After a serially inoculation passage in inbred mice homozygous for a single PRNP genotype, all the scrapie strains retained their original disease profile. These observations led investigators to question whether varied phenotypic strains were dominated by different conformation isoforms of same cellular prion precursor, a possibility suggested by conformation-dependent immunoassay (Safar 1998), or whether these strains were a result of various PrP^{Sc} associated molecules.

Many researchers have identified various nonprotein molecules that are bound to prion proteins. The precise biological and physiological roles remain the topic of further investigation. Copper and zinc have been demonstrated to bind to PrP^{C}. *In vitro*, these divalent metals may contribute to prion superoxide dismutase (SOD)-like activity. Such SOD-like activity and copper content are dramatically reduced in scrapie-infected brain (Wong 2001).

In addition, prion rods, composed mainly of insoluble aggregates of the N-terminally truncated prion protein (PrP 27-30) are found to be associated with 1,4-linked glucose units. Sphingolipids, polysaccharide and other membrane components were also found in prion aggregates (Appel 1999, Klein 1998). The interaction between prion protein and lipid membranes could play a role in PrP conversion. For example, the negatively charged lipid membrane-inserted conformation of PrP is richer in β-sheet structure while the binding of PrP to raft-like membranes induces the formation of α-helical structure (Sanghera 2002).

In early 1990's, Snow et al, studying Gerstmann-Sträussler-Scheinker syndrome, Creutzfeldt-Jakob disease and scrapie, have documented the association of sulfated proteoglygan to the prion protein amyloid plaques (Snow 1990). In an immunohistochemistry study using heparan sulfate antibodies (anti-HS) and heparan sulfate proteoglycan antibodies (anti-HSPG), McBride has demonstrated the correlation and association between HSPG and abnormal PrP in scrapie-infected mice brain. This correlation and association was observed as early as 70 days post-infection and throughout the course of the disease (McBride 1998). In *in vitro* conversion from PrP^{C} to PrP^{Sc} and in prion infectivity reconstitution experiments, sulfate glycans have been shown either to facilitate the conversion or to escalate infectivity (Wong 2001, Shaked 2001a). With recombinant GST::full-length prion and GST::prion fragment, Warner recently demonstrated direct binding of recombinant prion to heparin and heparan sulfate (Warner 2002). The peptide region 23-52 in prion sequence was positive in all HS and HSPG binding tests. Since the peptide failed to compete with full-length prion for binding to heparin, the author suggested that there might be another major GAG-binding site in intact PrP^{C}. Another noteworthy observation is that GAGs from different species (bovine and porcine) or from different organs (lung, kidney and intestine) have shown different affinities for prion binding. The difference in affinity may be due to prion sequence itself, or may depend on the presence of particular sugar unit in the tested GAGs.

Through a mechanism that is perhaps different from that by which glycans participate in the conversion of PrP^{C} to PrP^{Sc}, DNA could also convert cellular prion protein into β-sheet conformation (Cordeiro 2001). Nandi demonstrated that prion peptide 106-126 is the region that participated in the nucleic acid-prion complex association (Nandi 1998). Interestingly, not only was PK resistant amyloid aggregate obtained from the interaction between prion protein and nucleic acids, the nucleic acid morphology also changed to condensed globular structures, similar to nucleic acid structures induced by the HIV-1 NCp7 protein, but not to the structure induced by histones (Nandi 2001). Based on those *in vitro* conformation and conversion studies, it was hypothesized that DNA would act as a guardian of the PrP^{Sc} conformation as well as a catalyst to facilitate PrP^{Sc} conversion and aggregation (Cordeiro 2001).

Whether one accepts or rejects the "protein only" or "prion only" hypothesis, the effort to link inherited information to TSE disease or the search for genetic make up related to TSE disease has never stopped. The presence of a tightly bound RNA or DNA molecule in the prion particle was proposed to explain propagation of different strains of scrapie agent with distinct phenotypes in animals homozygous for the *PRNP* gene (Weissmann 1991). Analysis of highly purified scrapie prions by return refocusing gel electrophoresis revealed the small size of remaining nucleic acids, although the size of extracted

nucleotides was too small to encode any meaningful protein (Kellings 1992). In a recent report, however, Narang indicated that animals inoculated with ssDNA purified from scrapie-hamster brains mixed with non-pathogenic prion developed clinical disease (Narang 2002). Based on his findings, he postulated that the "accessory protein" coded by the ssDNA may be involved in PrP^{C} to PrP^{Sc} conversion. Although the role of nucleic acids in prion-associated disease is controversial, it is clear that PrP^{Sc} aggregates are tightly associated with these small molecules.

Infectivity and Transmissibility of Prion Diseases

Classic CJD in human has been grouped into three etiological types: sporadic (CJD), inherited (GSS or FFI), and acquired, which is very rare and includes diseases such as kuru and iatrogenic CJD. There is no hard evidence indicating any of CJD diseases is related to animal TSEs that may have crossed species barriers. The epidemic of kuru has provided the largest body of evidence of acquired human prion disease. Searching for risk factors and possible sources of infection in sporadic CJD patients revealed no significant correlation of disease to diet, blood transfusion or receiving other blood product. However, after intracerebral inoculation to mice, the infectivity in blood obtained from CJD patients indicated the possible presence of the CJD agent (Manuelidis 1985, Tateishi 1985).

BSE appears to have originated from dietary exposure. Nutritional supplements of processed meat and bone meal derived from scrapie disease infected carcasses were used to feed cattle livestock and other captive animals. In spite of BSE originating from scrapie, no case of de novo infection or cow-to-cow transmission has been reported.

There is mounting evidence, however, that links vCJD to BSE, The growing epidemiological data locates the majority of vCJD cases in UK where the overwhelming majority of BSE cases have also been reported. The link between vCJD and BSE is further supported by the neuropathologic evidence obtained from BSE-adapted macaques, the nearest model to humans (Bruce 1997), and from the study on inbred mice inoculated with the agent causing BSE and vCJD (Lasmézas 1996).

Although no vCJD patient has been documented as a victim of human-to-human transmission, the close link between BSE and vCJD attracted considerable attention. Concerns about human infection have been based on the observation that PrP^{Sc} is readily detectable in BSE and vCJD lymphoreticular tissues but not in classic CJD (Hill 1997), followed by the presumption that scrapie pathogen from sheep passage to cattle may have altered host range and become more adaptable to human. Experimental precedents for such behavior are well known: passage of mouse-adapted strains of scrapie through hamsters altered their transmissibility on back passage to mice (Kimberlin 1987, Kimberlin 1989); human strains of kuru or CJD did not transmit to ferrets or goats until passaged through primates or cats (Gibbs 1979); and a bovine strain of BSE did not transmit to hamsters until passaged through mice (Foster 1994). Alternatively, if BSE originated from a spontaneous mutation in cattle, experimental studies of species susceptibility to this new strain of transmissible spongiform encephalopathy (TSE) had not sufficiently advanced to predict that humans would not be susceptible.

In addition to CJD infectivity in blood described above, other TSE infectivity in blood has also been demonstrated in various experimental animals. Most blood for infectivity studies was obtained from TSE – adapted rodents such as mice and hamsters. The only exception was a study conducted in the sheep model. In this experiment, a sheep transfused with whole blood, taken from another sheep inoculated with BSE brain lysate, developed symptoms of BSE (Houston 2000, Hunter 2002). However, these experimental results yet need to be fully evaluated. The infectivity in blood has been established in rodent animals through intracerebral and intravenous transmission with mice-adapted BSE, mice-adapted vCJD and other rodent animal adapted TSE strains. Although the infectivity in lymphocyte-rich buffy-coat is greater than in plasma, it only accounts for relatively a small portion when compared to whole blood inoculums. The molecular definition of this infectious agent present in the blood is still under investigation. It is anticipated that finding of such infectious agent in blood would help us to better understand the relationship between PrP^{Sc} and TSE disease.

Study on human CJD and vCJD disease indicated that genomic susceptibility may yet be another factor that may influence the spread of TSE in humans. The majority of sporadic CJD patients were found to be homozygous for Met/Met or for Val/Val at codon 129 (Belay 1999). Nevertheless, all reported vCJD cases have been found to be homozygous for Met/Met.

The size and duration of vCJD epidemic still remains uncertain. Depending on the assumptions made and the modeling calculations employed, different predictions were proposed. One estimation of total vJCD predicts as few as 205 cases (Valleron 2001). On the other hand, another prediction for vCJD mortality for the next 80 years ranges from 50 to 50,000 if infection comes only from BSE. It could reach up to 150,000 if BSE is proven to infect sheep and if subsequently it is allowed to enter human food chain (Ferguson 2002). Although it is impossible to make accurate predictions if the necessary parameters are either mistaken or not available, one thing is certain that if vCJD infectivity is present in blood, any prediction will be an underestimate. In addition, vCJD has been proven to be a new disease entity and not simply the result of increased surveillance of CJD in humans (Hillier 2002).

Countermeasures have been taken by government to eliminate the spread of BSE incidence. Ruminant protein feed was banned in US and UK (1988). A series of measures have also been taken to prevent potentially infected meat from entering human food chain. To further reduce the human risk, FDA and CBER has issued a new policy in Aug. 2001, which indefinitely defers any human blood donor who stayed cumulative ≥ 6 month during 1980-1996 in the United Kingdom (FDA 2001).

Diagnostic Assay for Prion Disease

Clinical symptoms of prion disease often overlap with those of other neuronal degenerative diseases that make diagnosis difficult. So far, PK resistant PrP 27-30 is the only protein marker linked to TSE disease. Therefore, the detection of this agent has become the focus of assay development. However the development of monoclonal antibody specific for PrP^{Sc} was extremely difficult, not only because pathogenic PrP^{Sc} isoform and normal cellular PrP^{C} are two conformers of the same protein with an identical primary sequence, but also because the prion appears to be a weak immunogen. The only antibody reported to be able to recognize PrP^{Sc} specifically is not practically useful (Korth 1997). Other prion sequence-specific monoclonal and polyclonal antibodies are unable to distinguish PrP^{Sc} from PrP^{C}. Nevertheless, these antibodies (such as 3F4, 6H4 described in US4806627 and EP0861900.) are still commonly in use for capture or for detection of prion protein in combination with sample treatment and separation techniques to isolate PrP^{Sc} from PrP^{C} (Korth 1997, Kascsak 1987).

Since the outbreak of BSE in 1986, all commercially available tests for prion disease use, as their sample source, tissues taken from postmortem animals and humans. Among those, a tissue homogenate-based PrP^{Sc} assay, referred to as DELFIA (dissociation-enhanced lanthanide fluoroimmunoassay), was developed for the detection of scrapie prion (Barnard 2000 and a method described in US20020137114A1). It requires a protein denaturation step using GdnHCl, in combination with optional sample PK treatment and PrP^{Sc} enrichment by sodium phosphotungstic acid (NaPTA) precipitation. Since the transformation of PrP^{C} to PrP^{Sc} is accompanied by the burial of epitopes near the N terminus of PrP, in DELFIA, monoclonal antibodies directed against the N-terminus of PrP are used to measure the difference of mAb binding affinity to the α-helical and β-sheet conformations before and after PrP denaturation (Peretz 1997). Another conformational-dependent immunoassay (CDI) combined with ELISA and fluorescence detection (Safar 1998, US 20010001061A1, US20020001817A1) was described in conformation studies in PrP^{Sc} strains.

In a tissue distribution study of PrP^{Sc} in vCJD patients, an improved NaPTA precipitation was described to enrich PrP^{Sc} from brain and from other peripheral tissue homogenates (Wadsworth 2001). The modification employed endonuclease treatment to reduce sample viscosity prior to NaPTA precipitation. The recovery of PrP^{Sc} in the precipitated pellet was reported to be consistently greater than 90% while recovery of PrP^{C} was about 5%. After PK digestion, the presence of PK resistant prion was verified in Western blot using 3F4 monoclonal antibody.

In another similar immunoblot assay, PK digestion was also used to eliminate PrP^C. 6H4 was then used to determine the presence of PrP^{Sc} (Schaller 1999). Based on this first generation assay, a second-generation luminescence immunoassay was developed in which 6H4 was coated on plates as a capture antibody. The horseradish peroxidase (POD)-conjugated detection antibody used was a mouse monoclonal anti-PrP antibody, able to form a complex with PrP27–30 bound to 6H4 (Biffiger 2002).

The European Commission in 1999 evaluated 4 BSE test kits from different manufacturers (Moynagh 1999). They all used bovine brain tissue as a sample source, and all required a separate sample preparation procedure. Depending on the kit instructions, the brain tissue homogenate needed to be processed, including denaturation, PK digestion or PrP^{Sc} enrichment. The assay detection systems employed in DELFA, immunoblot, or in plate ELISA formats used either chemiluminescent or a colorimetric substrate.

In order to control the spread of the disease in the absence of a live-animal screening test, an extensive slaughter of cattle was carried out once an affected animal was identified within a herd. The urgency for a live animal diagnosis assay was reinforced when the first cases of variant Creutzfeldt-Jakob disease was reported in 1996.

Antemortem TSE diagnosis development presents three major difficulties: (1) insufficient sensitivity – Except in brain tissue, PrP^{Sc} concentrations in other tissues or fluids is considered to be very low. Therefore, a highly sensitive technique is required for detection. (2) Appropriate sample treatment – Any protein denaturation or PK digestion process may have a potential impact on pathogenic PrP^{Sc} structure, with the possibility of causing a false negative result. For example, it has been suggested that an intermediate form of PrP^{Sc} may not be PK resistant (Horiuchi 1999, Jackson1999, Swietnicki 2000). And (3), the lack of PrP^{Sc}-specific antibodies and the incompletely characterized molecular relationship between the pathogenic agent and PrP^{Sc} in blood make it difficult to design an assay format for antemortem diagnosis.

A possible approach to boost the sensitivity is in-vitro amplification of PrP^{Sc}. It has been reported that when PrP^{Sc} was present, repetitive cycles of sonication could induce protease-sensitive cellular PrP to form protease resistant aggregates. The authors explained that in this "protein-misfolding cyclic amplification" (PMCA) process, sonication could disrupt newly formed aggregates and generate multiple smaller units for the continued formation of new PrP^{Sc} (Saborio 2001, WO0204954). At the end of 40 PMCA cycles, the sample was subjected to PK digestion and detected by immunoblot. It claimed that the amplification generated more than 30-fold protease resistant PrP. Since proteinase resistant PrP were generated at the expense of the normal prion protein as substrate through amplification cycles, a large quantity of same-species normal prion was required. It has not been demonstrated whether normal prion from another species could also work as substrate, or prion protein from a recombinant source or from sources other than brain tissue could be used. Such evidence would be useful when detection of vCJD is desired.

Immunchistochemistry of third eyelid lymphoid tissue has been described for preclinical diagnosis of ovine scrapie (O'Rourke 2000, US6165784, US6261790). Relying on a small surgical procedure, the assay makes use of sheep peripheral tissue, the third eyelid lymphoid for scrapie detection. The immunohistochemistry used a cocktail of pan-specific monoclonal antibodies to differentiate one isoform from the other. Following formalin fixation to reduce PrP^C reactivity, the sample is subjected to formic acid and heat pretreatments which enhance the PrP^{Sc} reactivity. In spite of the fact that the assay is still tissue based and the observation that PrP^{Sc} displayed poor immunoreactivity in immunohistochemistry staining unless treated with denaturing agents, this antemortem preclinical diagnosis has made a step towards live-animal test as well as provided a way of identification of scrapie-affected sheep during the early, preclinical stage of scrapie.

In addition to the traditional identification of pathogenic prion by eliminating cellular prion followed by non-discriminatory anti-prion antibody recognition, other reagents were found to be able to differentiate PrP^{Sc} from PrP^C, such as plasminogen and fibrinogen. The mechanism of interaction between these human

blood component proteins and PrP^{Sc} is not clear. However, when immobilized on magnetic beads, plasminogen selectively precipitated PrP^{Sc} from brain homogenates of mouse, human, cattle and sheep. The evidence provided suggested that a property common to PrP^{Sc} of various species, rather than prion primary sequence or the specific tertiary structure of individual PrP^{Sc} molecules, could be responsible for binding to plasminogen (Fischer 2000, Maissen 2001). The application for the use of plasminogen and other serum/plasma proteins for the capture and detection of pathogenic prion protein has been described in WO0200713 and in US20010053533A1 (Aguzzi 2001).

Recent investigations have identified a new isoform of the prion protein in the urine of animals and humans with prion disease (Shaked 2001b, WO0233420A2). This isoform, referred to as $UPrP^{Sc}$ by the investigators, was precipitable, PK resistant, and detectable only in infected individuals but not in normal controls. Most importantly, as indicated in their publication, $UPrP^{Sc}$ appeared long before the clinical signs developed in inoculated hamsters. However, when $UPrP^{Sc}$ isolated from scrapie hamster urine was inoculated back in normal hamster intracerebrally, it did not cause disease even after 270 days, well beyond the incubation period in which animal would develop clinical signs if comparable amount of brain derived PrP^{Sc} had been inoculated. It is not impossible that those hamsters, inoculated intracerebrally with $UPrP^{Sc}$, were still in a subclinical or carrier state. Moreover, PK-resistant PrP was not found in the kidneys, which implies that this $UPrP^{Sc}$ could have originated from other organs and been transported to the urine via the blood. This important observation will undoubtedly lead to a better understanding of PrP metabolism.

Therefore there remains an unmet need for a better way to detect PrP^{Sc} and diagnose TSE in humans and animals. The aim of the present invention is to provide a non-intrusive way to isolate, concentrate and monitor the TSE disease-related pathogenic prion protein. The invention, including the use of selective anti-DNA antibody to bind the PrP^{Sc} through recognition of an associated binding partner, involves the discriminatory capture of PrP^{Sc} but not cellular prion protein. We provide evidence of a high affinity association of nucleic acid to PrP^{Sc}, and we demonstrate that such nucleic acids::PrP^{Sc} complex survived even after PK digestion and nuclease treatment.

Summary of the Invention

The evidence provided in support of this invention demonstrated that PrP^{Sc} is associated with high affinity to nucleic acid, mainly DNA as investigated. A similar association with nucleic acid was not observed with normal cellular PrP^{C}. The evidence also demonstrated that the association was strong, resistant to PK digestion and nuclease treatment, and that PrP^{Sc} could be readily isolated by selective anti-DNA antibodies.

This invention relates to the use of anti-DNA antibodies to capture PrP^{Sc} through nucleic acids associated with high affinity to PrP^{Sc}, in combination with any prion sequence-specific antibody for the detection of PrP^{Sc}.

In another aspect, this invention relates to the selective DNA antibody, as described above, that preferably binds to pathogenic prion protein but not to the normal cellular form of prion protein.

In another aspect, this invention relates to the selective DNA antibody, as described above, for the detection of PrP^{Sc} through high affinity recognition of associated nucleic acids in combination of prion sequence specific antibodies.

In another aspect, this invention relates to the selective DNA antibody, as described above, for the isolation, purification, capture, elimination and monitoring PrP^{Sc} in biological reagent production.

In another aspect, this invention relates to compositions and kits for determining the presence of PrP^{Sc}, comprising anti-DNA antibody, as described above, for either capture or for detection step in the assay procedure.

In another aspect, this invention relates to compositions and kits for determining the presence of PrP^{Sc} antibody produced in response to high affinity associated DNA as a binding partner to pathogenic prion protein.

In yet another aspect, this invention relates to anti-PrP^{Sc} antibodies and their production using the said nucleic acids that can interact with and/or associate to PrP^{sc}, and their use in detecting nucleic acid::PrP^{Sc} complex and prion disease infection.

In another aspect, this invention relates to a non-harsh sample treatment procedure involving nuclease digestion for the benefit of the use of selective DNA antibody as described above.

Some examples of specific embodiments of the invention are as follows:

A method for discriminating between infectious and noninfectious prions comprising:
first contacting a sample with an anti-nucleic acid antibody,
then adding a prion specific antibody to form a complex between the anti-nucleic acid antibody, prion and prion specific antibody, and
detecting the complexes.

A method for diagnosing transmissible spongiform encephalopathies in a patient comprising:
drawing a sample from a patient,
contacting a sample with an anti-nucleic acid antibody,
then adding a prion specific antibody to form a complex between the anti-nucleic acid antibody, prion and prion specific antibody, and
detecting the complexes, whereby detecting the complexes provides and indication of transmissible spongiform encephalopathies in a patient.

An immunoassay for detecting infectious prions comprising:
providing a solid support having bound thereto an anti-nucleic acid antibody,
contacting the solid support with a sample,
washing the support to remove any unbound sample,
contacting the solid support with a prion specific antibody, and
carrying out a detection step to determine if prions are bound to the solid support.

A kit for the detection of infectious prions comprising
a solid support having bound thereto an anti-nucleic acid antibody, and
a labeled prion specific antibody.

An immunoassay for detecting infectious prions comprising:
providing a solid support coated with an agent to bind an anti-nucleic acid antibody
contacting the solid support with a sample,
washing the support to remove any unbound sample,
contacting the solid support with a prion specific antibody, and
carrying out a detection step to determine if prions are bound to the solid support.

Another embodiment of the immunoassay described above provides a solid support coated or carrying an agent that is capable of binding the anti-nucleic acid antibody. For example, using avidin or streptavidin on the solid support and biotinylating the anti-nucleic acid antibody so that it binds to the solid support via the avidin or streptavidin.

A further embodiment of the invention is directed to a vaccine composition comprising anti-nucleic acid antibodies and a pharmaceutically acceptable carrier.

Use of a therapeutically effective amount of a vaccine composition comprising anti-nucleic acid antibodies and a pharmaceutically acceptable carrier for treating prion disease in a patient.

Use of a therapeutically effective amount of a vaccine composition comprising anti-nucleic acid antibodies and a pharmaceutically acceptable carrier for inducing neutralized infectious prions in a patient susceptible to or suffering from a prion disease.

A method of treating a prion disease in a patient comprising administering a therapeutically effective amount of a vaccine composition comprising anti-nucleic acid antibodies and a pharmaceutically acceptable carrier.

A method of inducing neutralized infectious prions in a patient susceptible to or suffering from a prion disease comprising administering a therapeutically effective amount of a vaccine composition comprising anti-nucleic acid antibodies and a pharmaceutically acceptable carrier.

Brief Description of the Drawings

Figure 1: anti-DNA IP capture and immunoblot of scrapie hamster PrP^{Sc}. Hamster scrapie PrP^{Sc} was immunocaptured by anti-DNA antibodies followed by SDS-PAGE and 3F4 immunoblot. Results were obtained from three separate experiments (Lane# 1-4, 5-8 and 9-10).

Figure 2: IP capture and immunoblot of BSE PrPSc. BSE PrP^{Sc} was detected by anti-DNA antibodies and DNA binding protein through immunoprecipitation (IP) followed by 6H4 immunoblot. Results were obtained from two separate experiments (Lane# 1-11, and 12-14).

Figure 3: IP and immunoblot of hamster prion with various treatment. IP and immunoblot were performed under various conditions for each anti-DNA antibody. (1) Standard: IP performed in IP buffer with scrapie or normal hamster brain homogenate (lane 1, 5, 9,13,17-20). (2) DNA inhibition: 5 ug/mL phenol-chloroform extracted, ethanol precipitated, and sonicated Salmon DNA (Sigma, MO, USA, Cat.# D7656) was added in the IP buffer as inhibitor (lane 2, 6,10,14). (3) Proteinase K digestion: scrapie hamster brain homogenate was treated with Proteinase K at 50ug/mL at 37C for 1 hour. Digestion was stopped by adding Pefablc SC to a final of 4mM. The digested homogenate was spiked in IP buffer followed by standard IP (lane 3, 7,11,15). (4) Nuclease digestion: scrapie hamster brain homogenate was treated with Benzonase® nuclease at 100U/mL at 37C for 1 hour. Digestion was stopped by adding EDTA to a final of 10mM. The digested homogenate was spiked in IP buffer followed by standard IP (lane 4, 8,12,16).

Figure 4. Immunocapture of PrP^{Sc} from brains of sCJD and vCJD by OCD4. The OCD4 conjugated beads were used to immunoprecipitate PrP in clarified brain homogenates from patients affected by either sCJD or vCJD, and two unaffected subjects (normal controls). The immunoprecipitates were then analyzed by SDS-PAGE (12% gel) and Western blotting using the anti-PrP antibody 3F4. OCD4 specifically captures PrP^{Sc} in brains of sCJD (lane 1) and vCJD (lane 3) but not PrP^{C} in normal brains (lanes 5 and 7). OCD4 captured PrP from sCJD and vCJD brains are authentic PrP^{Sc} since treatment with PK (50 ug/ml for 1 h at 37 °C) generates the PK-resistant core PrP^{res} fragments (lanes 2 and 4). ***Right panel.*** The clarified brain homogenates from sCJD, vCJD and normal controls were incubated in the absence (-) or presence (+) of PK (50 ug/ml for 1 h at 37 °C). After the PK digestion was terminated, samples were directly loaded onto SDS-PAGE gels (12%) and analyzed on Western blots using the 3F4 antibody. Brain tissues of sCJD type 2 and vCJD are well-characterized TSE reference materials from WHO.

Figure 5. The OCD4 based capture immunoassay for PrP^{Sc} in brains of vCJD patients. Brain homogenates (10 ul) from 10 cases of vCJD (v1-v10) were used in the OCD4/3F4 capture immunoprecipitation assay. The experiment was conducted at the NCJDSU, UK, using kindly provided vCJD cases (case numbers: 95/052(1), 96/045(2), 97/049(3), 98/063(4), 98/148(5), 98/154(6), 99/082(7), 99/090(8), 00/066(9), 00/101(10)).

Figure 6. Immunocapture of both the full-length PrP^{Sc} and the PK-resistant core fragments by OCD4. Aliquots of brain homogenates from three vCJD cases were used in this experiment and conducted at NCJDSU, UK. The first aliquot was digested with PK (+PK). The second aliquot was digested with PK and then was subjected to immunoprecipitation by the OCD4 (+PK +OCD4). The third aliquot underwent direct immunoprecipitation by OCD4 without the PK treatment (-PK+OCD4). All above

samples were analyzed on Western blots using the 3F4 antibody. The PK-res PrP fragments (lanes 1-5) were recovered in the OCD4 immunoprecipitates (lanes 6-10). The full-length PrP^{Sc} in untreated samples could be efficiently captured by OCD4 as well (lanes 11-15). Note that the input volume of the 10% brain homogenates was varied in some cases for better resolution of the PrP bands.

Figure 7. OCD4 Immunocapture of PK-resistant PrP in cVJD spleen. Spleen lysate was prepared from a case of vCJD (case number 96/045 and 98/148, provided by NCJDSU at Edinburgh) using 10 % homogenate followed by brief centrifuge to remove debris. PK treatment was performed at 50 ug/ml for 1 h at 37°C before the reaction was terminated by 10 mM Pefabloc. Aliquots of each 100 ul of digested spleen lysate were either pelleted by centrifugation at 14,000Xg for 1 h at 4°C (lane 1), or were subjected to immunoprecipitation with OCD4 (lane 2 and 4) and with an unrelated mAb (lane 3 and 5, as control for non-specific binding). Detection of PK-resistant PrP in spleen was done on Western blots using the 3F4 antibody.

Figure 8. OCD4 immunocapture of BSE PrP^{Sc}. Brain lysates (10%, w/v) from either BSE or bovine control (CON) were prepared by homogenization in lysis buffer followed by brief centrifugation. Aliquots of clarified brain lysates (1 ul each) were used in immunoprecipitation (IP) before (lanes 1 and 2) or after (lanes 3 and 4) PK digestion (50 ug/ml for 1 h at 37°C). Additional aliquots (1 ul) without IP and PK digestion (lanes 5 and 6) served as reference for total input. All samples were then separated by SDS-PAGE and were detected on Western blots using the 6H4 antibody capable of recognizing bovine PrP. OCD4 immuoprecipitated PrP only from BSE brain in the absence or presence of PK. This experiment was conducted at the P3 facility of the Veterinary Laboratories Agency in Waybridge, London where brain tissues of BSE-affected and normal cattle were kindly provided.

Figure 9. OCD4 Immunocapture of PrP^{Sc} in scrapie sheep. Immunoprecipitation by OCD4 of PrP from brains of natural scrapie (Sc) and normal sheep control (N) before and after treatment with PK (50 ug/ml for 1 h at 37°C). Experiments were performed as described in the main text. Immunoprecipitates on the OCD4 conjugated beads were probed on Western blots with the 6H4 antibody on Western blots. Each assay used 1 ul of 10% brain homogenates.

Figure 10. Effective immunocapture of spiked PrP^{Sc} in human plasma by OCD4. The spike material (S) used was 1uL of 5 % homogenate of scrapie hamster brain. Standard PrP^{Sc} immunocapture in IP buffer (1mL) was shown in lanes 1 and 8. For lanes 2-4, the spike (1uL) was added to 0.6ml of three normal human plasma preparations A, B, and C (0.6ml plasma/S) with the addition of 400uL IP buffer. Lanes 5-7 represent the non-spiked plasma aliquots (0.6ml plasma) with 0.4mL IP buffer. For lanes 9-11, 5mL each of plasma A, B, and C were preincubated with the OCD4 beads followed by brief washes of the beads in PBS. The plasma treated beads were then incubated with the spike (1uL) in 1 mL of IP buffer (5mL plasma→S). Standard immunoprecipitation by the OCD conjugated beads was done in a total volume of 1mL followed by Western blotting using the 3F4 antibody as described in the main text. As compared to the input control (lanes 1 and 8), significant recapture of spiked PrP^{Sc} by OCD4 was achieved in plasma present in large excess (600uL plasma vs. 1uL spike) (lanes 2-4). Moreover, the fact that preincubation of OCD4 conjugated beads with large volume of normal plasma did not compromise or block its binding ability to capture PrP^{Sc} as indicated in lane 9-11, exclude the possibility of potential OCD4 inhibitors present in human plasma. OCD4 did not capture PrP^{C} from human plasma (lanes 5-7).

Detailed Description of the Invention

The term "sample" as used herein, refers to any substance, which may contain the analyte of interest. A sample can be biological fluid, such as whole blood or whole blood components including red blood cells, white blood cells, platelets, serum and plasma, ascites, urine, cerebrospinal fluid, and other constituents of the body which may contain the analyte of interest, such as brain homogenate. Optionally, samples

may be obtained from water, soil, and vegetation. The term "patient" as used herein, refers to humans and/or animals.

Various immunoassay protocols are known and could be applied to the present invention. The assay can be carried out using any enzyme label which can be attached to the anti-prion antibody to form a labelled ligand. Enzymes such as oxidases, e.g., glucose oxidase, peroxidases, e.g., horseradish peroxidase (HRP), alkaline phosphatase and galactosidases are preferred labels. It is within the skill of one of ordinary skill in the art to determine a suitable substrate for a given label. The substrate can be a material which is directly acted upon by the enzyme label or a material that is involved in a series of reactions which involve enzymatic reaction of the label. Other labels and means for detection could be for example, a ligand, nucleotide, or biotin. Detection of the labeled antibody could be by various methods including enzyme amplification with polymeric conjugates and immuno PCR.

The following examples are given to illustrate but not limit the scope of the invention.

Brain homogenate preparation:

Normal and scrapie hamster brain lysate were obtained from Baltimore Research and Education Foundation as 10% whole brain tissue homogenate in PBS (w/v). The lysate was further treated by adding 1/10 volume of 10X detergent homogenate buffer, composed of 5% sodium deoxycolate and 5% Igpal CA-630 (equivalent to NP-40) in PBS, incubating at 4C for 1hr., followed by centrifugation at 1000g for 10 minutes. The resulting supernatant was collected and used in the assay.

Normal and BSE bovine brain tissue were provided by Veterinary Laboratories Agency (VLA), UK. Normal and scrapie sheep brain tissue were provided by Animal Disease Research Unit of USDA, USA. Normal human brain tissue were provided by National Prion Disease Pathology Surveillance Center (NPDPSC), USA. Human sCJD and vCJD brain tissue were provided by NPDPSC and National CJD Surveillance Unit (NCJDSU), UK. Brain tissue was processed the same way (or similar) as hamster brain homogenate preparation.

Anti-DNA antibodies and DNA binding protein:

Monoclonal antibodies obtained from commercial sources were (1) murine monoclonal antibody recognizing ss-, ds- DNA, subclass IgM, Cat# 12403 and subclass IgG2b, Cat# 12404 from QED Bioscience, (2) murine monoclonal antibody recognizing ds- DNA, clone AE-2, subclass IgG3, Cat# 2660-2308 and murine monoclonal antibody recognizing ss-, ds-, clone 49/4A1, subclass Ig2b, Cat# 2660-2316 from Biogenesis. The immunogens used to raise these antibodies were Calf thymus DNA and nuclei from Raji Burkitts lymphoma Cells as indicated by manufactures. Additional monoclonal antibodies from other than commercial source were also evaluated. Single Stranded Binding Protein (SSB) from E.coli purchased from Sigma (Sigma, MO, USA, Cat.# S3917).

Conjugation of antibody and protein to magnetic beads:

0.35 mL Dynabeads® M-280 Tosylactivated (Dynal Biotech, NY, USA, Cat.# 142.03/04) were washed twice with PBS and the beads isolated from buffer with the magnet (Dynal Magnetic Particle Concentrator, MPC). 100 ug of purified antibody or protein in 1mL PBS was added to the washed beads. Incubation with rotation was performed at 37C for 18-20 hours. The beads were isolated from the buffer with the MPC, washed twice with 1 ml PBS (0.1% BSA), and rotated for 5 minutes at room temperature while washing. The antibody-conjugated beads were then blocked for 3-4 hours, 37°C with 0.2 M Tris-HCl, pH 8.0, containing 0.1% BSA. The beads were subsequently washed 2 times with 1 ml PBS (0.1% BSA) and once with 1 ml PBS (0.1% BSA, 1% Tween 20) incubating each time for 10 minutes at room temp. The beads were then washed once with 1 ml PBS (0.1%BSA) and then stored in 1ml PBS (0.05% sodium azide) at 4°C.

Proteinase K digestion and Benzonase® Nuclease digestion:

Conditions for the PK digestion of brain lysate: Brain homogenate was suspended in PBS buffer with or without non-ionic detergent. The total homogenate protein concentration was no more than 2.5 mg/mL. PK (Roche Diagnostics, IN, USA, Cat.# 1373196) was added to a final concentration of 50ug/mL.

Incubation was at 37C for 0.5 to 1 hour. Digestion was stopped by adding Pefabloc SC (Roche Diagnostics, IN, USA, Cat.# 1585916) to a final concentration of 4mM.

Conditions for the Benzonase® Nuclease digestion of brain lysate: Brain homogenate was suspended in Tris-HCl buffer, with or without non-ionic detergent, containing 2mM Mg^{++}. Total homogenate protein concentration was no more than 2.5 mg/mL. Nuclease (CN Biosciences, CA, USA, Cat.# 70664) was added to a final concentration of 100U/mL. Incubation was at 37C for 0.5 to 1 hour. Digestion was stopped by adding EDTA to a final concentration of 10mM.

Immunoprecipitation (IP), non-reducing electrophoresis and immunoblot detection of PrP^{Sc}:
Anti-DNA antibody conjugated magnetic beads were used to capture PrP^{Sc} from brain homogenate by immunoprecipitation. The IP procedure consists of the following protocol: mix 100uL antibody conjugated beads with 1-5 uL of brain homogenate in a total of 1 mL IP buffer (3% Tween20 and 3% Igpal CA-630 in PBS) and incubate at 25C for 2.5 hours with rotation → Separate beads using MPC device and wash beads 3 times of 30 second vortexing with IP wash buffer (2% Tween20 and 2% Igpal CA-630 in PBS) → Elute captured PrP^{Sc} by heating beads with NuPAGE sample buffer for 10-15 minutes. The eluted sample from IP capture were loaded onto a 4-12% NuPAGE® Bis-Tris Gel (Invitrogen, CA, USA, Cat.# NP0302) and subjected to non-reducing electrophoresis at 200V for 45 minutes. The immunoblot procedure was perfomed as follows: transfer separated proteins in the gel to a 0.2um PVDF membrane (Invitrogen, Cat# LC2002) at 30V for 60 minutes → Block the membrane with Blocker™ Casein in TBS (0.05% Tween20) (Pierce Chemical Corp., IL, USA, Cat.# 37532) either at 25C for 1 hour with shaking or at 4C overnight. → As primary antibody, use 3F4 (Signet, MA, USA, Cat.# 9620-02) at 1:3000 dilution to detect hamster and human PrP^{Sc} or use 6H4 (Prionics AG, Switzerland, Cat.# 01-011) at 1:5000 dilution or to detect bovine and sheep PrP^{Sc} respectively. Incubate the membrane with diluted primary antibody in 10% Blocker™ Casein in TBST buffer (25mM Tri-Cl, 0.2M NaCl, 0.2% Tween20, pH 8.0) at 25C for 1 hour with shaking. → Wash 3X 5minutes with TBST buffer with shaking. → Incubate membrane with horseradish peroxidase conjugated goat polyclonal anti-mouse IgG (H+L) (Jackson ImmunoResearch Laboratories, PA, USA, Cat.# 115-035-003) at 1:10,000 to 1:30,000 dilution in 50% Blocker™ Casein in TBST buffer at 25C for 1 hour with shaking. → Wash 6X 5minutes with TBST buffer with shaking. → Add ECL chemiluminescence substrate (Amersham Biosciences, NJ, USA, Cat.# RPN2109) or SuperSignal West Dura chemiluminescence substrate (Pierce) on membrane to develop for 5 minutes. → Take image by exposure either to Bio Max MR-2 film (Kodak, NY, USA) or to the ChemiDoc Gel Documentation System (Bio-Rad, CA, USA).

Vaccine and Therapetic Uses
Another aspect of the invention is directed toward therapeutic uses of the anti-nucleic acid antibodies as a therapeutic use. Animal models can be infected, for example with vCJD. One skilled in the art would then inject the animal with anti-nucliec acid antibodies in order to bind and neutralize the infectious prions. The result would be a reduction or elimination of the disease.

Advantages.
The present invention uses anti-DNA to capture PrP^{Sc} by recognition of high affinity associated nucleic acid in the nucleic acid::PrP^{Sc} complex. Because the tight association of nucleic acid only to PrP^{Sc} and not to PrP^{C}, the present invention provided a non-intrusive means for the detection of PrP^{Sc} while no PK digestion or other protein modification procedure required. It is anticipated that the mild conditions will preserve the original structure and conformation of the pathogenic prion protein, thereby offering opportunity to determine the presence of true PrP^{Sc} while minimizing the generation of PrP^{Sc} due to harsh sample treatment.

Provided evidence that Benzonase nuclease digestion does not compromise selective anti-DNA binding to nucleic acid::PrP^{Sc}, including limited endonuclease treatment in sample preparation or comprised in sample buffer could eliminate the interference of endogenous nucleic acid interference.

The use of anti-DNA antibodies offer advantages in that they display the binding specificity but can also be easily handled in direct coating to a solid phase as well as be conjugated to link to signal given reagents such as horseradish peroxidase (HRP), or to be adopted into other desired diagnosis assay format.

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(RXX) Bons N, Lehmann S, Mestre-Frances N, Dormont D, Brown P. (2002) Transfusion May;42(5):513-6 Brain and buffy coat transmission of bovine spongiform encephalopathy to the primate Microcebus murinus.

We Claim:

1. A method for discriminating between infectious and noninfectious prions comprising:
first contacting a sample with an anti-nucleic acid antibody,
then adding a prion specific antibody to form a complex between the anti-nucleic acid antibody, prion and prion specific antibody, and
detecting the complexes.

2. A method for diagnosing transmissible spongiform encephalopathies in a patient comprising:
drawing a sample from a patient,
contacting a sample with an anti-nucleic acid antibody,
then adding a prion specific antibody to form a complex between the anti-nucleic acid antibody, prion and prion specific antibody, and
detecting the complexes, whereby detecting the complexes provides and indication of transmissible spongiform encephalopathies in a patient.

3. An immunoassay for detecting infectious prions comprising:
providing a solid support having bound thereto an anti-nucleic acid antibody,
contacting the solid support with a sample,
washing the support to remove any unbound sample,
contacting the solid support with a prion specific antibody, and
carrying out a detection step to determine if prions are bound to the solid support.

4. A kit for the detection of infectious prions comprising
a solid support having bound thereto an anti-nucleic acid antibody, and
a labeled prion specific antibody.

5. An immunoassay for detecting infectious prions comprising:
providing a solid support coated with an agent to bind an anti-nucleic acid antibody
contacting the solid support with a sample,
washing the support to remove any unbound sample,
contacting the solid support with a prion specific antibody, and
carrying out a detection step to determine if prions are bound to the solid support.

6. The immunoassay claimed in claim 5 wherein the agent is avidin or streptavidin and the anti-nucleic acid antibody has been biotinylated.

7. A vaccine composition comprising anti-nucleic acid antibodies and a pharmaceutically acceptable carrier.

8. A method of treating a prion disease in a patient comprising administering a therapeutically effective amount of a vaccine composition comprising anti-nucleic acid antibodies and a pharmaceutically acceptable carrier.

9. A method of inducing neutralized infectious prions in a patient susceptible to or suffering from a prion disease comprising administering a therapeutically effective amount of a vaccine composition comprising anti-nucleic acid antibodies and a pharmaceutically acceptable carrier.

10. Use of an anti-nucleic acid antibody for diagnosing transmissible spongiform encephalopathies in a patient.

11. The use according to claim 10, wherein prion specific antibodies are also used.

12. Use of a therapeutically effective amount of a vaccine composition comprising anti-nucleic acid antibodies and a pharmaceutically acceptable carrier for treating prion disease in a patient.

13. Use of a therapeutically effective amount of a vaccine composition comprising anti-nucleic acid antibodies and a pharmaceutically acceptable carrier for inducing neutralized infectious prions in a patient susceptible to or suffering from a prion disease.

Application number numéro de demande: 2452946

Figures: 1,2,3,4,5,6,7,8,9,10

Pages:

Unscannable items
received with this application
(Request original documents in File Prep. Section on the 10th floor)

Documents reçu avec cette demande ne pouvant être balayés
(Commander les documents originaux dans la section de préparation des dossiers au 10ème étage)